



Full wwPDB X-ray Structure Validation Report ⓘ

Mar 10, 2018 – 04:55 am GMT

PDB ID : 1C4F
Title : GREEN FLUORESCENT PROTEIN S65T AT PH 4.6
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Deposited on : 1999-08-21
Resolution : 2.25 Å(reported)

This is a Full wwPDB X-ray Structure Validation Report for a publicly released PDB entry.

We welcome your comments at validation@mail.wwpdb.org

A user guide is available at

<https://www.wwpdb.org/validation/2017/XrayValidationReportHelp>

with specific help available everywhere you see the ⓘ symbol.

The following versions of software and data (see [references ⓘ](#)) were used in the production of this report:

MolProbity : 4.02b-467
Mogul : 1.7.3 (157068), CSD as539be (2018)
Xtriage (Phenix) : 1.13
EDS : trunk30967
Percentile statistics : 20171227.v01 (using entries in the PDB archive December 27th 2017)
Refmac : 5.8.0158
CCP4 : 7.0 (Gargrove)
Ideal geometry (proteins) : Engh & Huber (2001)
Ideal geometry (DNA, RNA) : Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP) : trunk30967

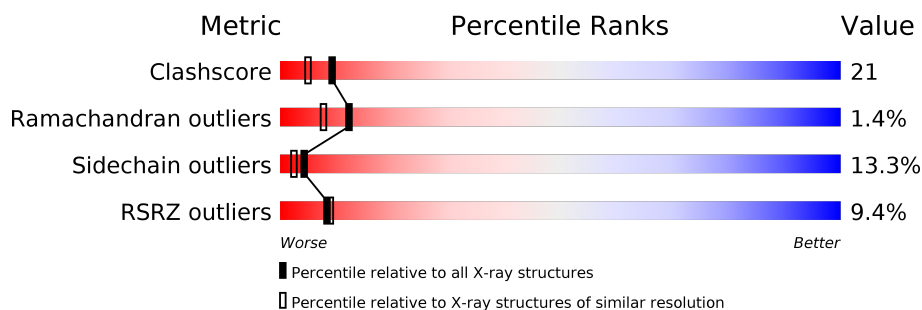
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

X-RAY DIFFRACTION


The reported resolution of this entry is 2.25 Å.

Percentile scores (ranging between 0-100) for global validation metrics of the entry are shown in the following graphic. The table shows the number of entries on which the scores are based.



Metric	Whole archive (#Entries)	Similar resolution (#Entries, resolution range(Å))
Clashscore	122126	1286 (2.26-2.26)
Ramachandran outliers	120053	1253 (2.26-2.26)
Sidechain outliers	120020	1254 (2.26-2.26)
RSRZ outliers	108989	1158 (2.26-2.26)

The table below summarises the geometric issues observed across the polymeric chains and their fit to the electron density. The red, orange, yellow and green segments on the lower bar indicate the fraction of residues that contain outliers for ≥ 3 , 2, 1 and 0 types of geometric quality criteria. A grey segment represents the fraction of residues that are not modelled. The numeric value for each fraction is indicated below the corresponding segment, with a dot representing fractions $\leq 5\%$. The upper red bar (where present) indicates the fraction of residues that have poor fit to the electron density. The numeric value is given above the bar.

Mol	Chain	Length	Quality of chain
1	A	236	

2 Entry composition

There are 2 unique types of molecules in this entry. The entry contains 1777 atoms, of which 0 are hydrogens and 0 are deuteriums.

In the tables below, the ZeroOcc column contains the number of atoms modelled with zero occupancy, the AltConf column contains the number of residues with at least one atom in alternate conformation and the Trace column contains the number of residues modelled with at most 2 atoms.

- Molecule 1 is a protein called GREEN FLUORESCENT PROTEIN.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	225	Total	C	N	O	S	18	0	0
			1715	1107	286	316	6			

There are 4 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	66	CRO	SER	CHROMOPHORE	UNP P42212
A	66	CRO	TYR	CHROMOPHORE	UNP P42212
A	66	CRO	GLY	CHROMOPHORE	UNP P42212
A	80	ARG	GLN	ENGINEERED	UNP P42212

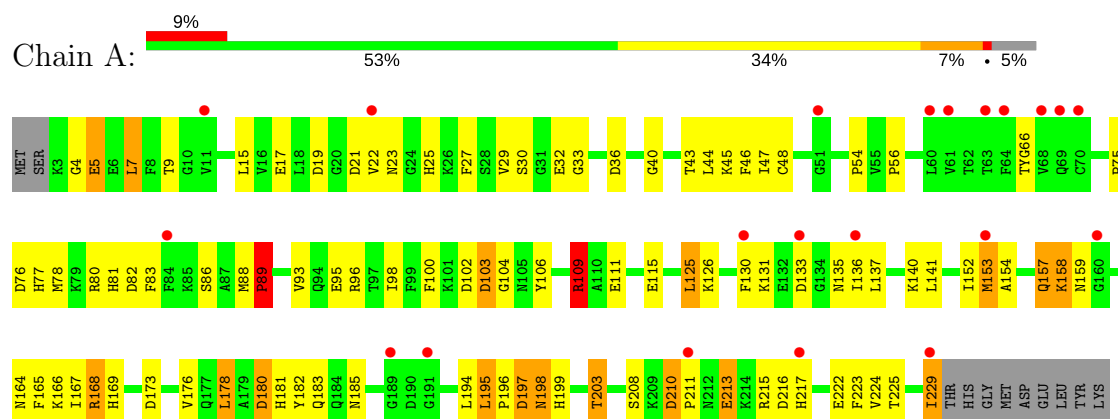
- Molecule 2 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
2	A	62	Total	O	0	0
			62	62		

3 Residue-property plots

These plots are drawn for all protein, RNA and DNA chains in the entry. The first graphic for a chain summarises the proportions of the various outlier classes displayed in the second graphic. The second graphic shows the sequence view annotated by issues in geometry and electron density. Residues are color-coded according to the number of geometric quality criteria for which they contain at least one outlier: green = 0, yellow = 1, orange = 2 and red = 3 or more. A red dot above a residue indicates a poor fit to the electron density ($RSRZ > 2$). Stretches of 2 or more consecutive residues without any outlier are shown as a green connector. Residues present in the sample, but not in the model, are shown in grey.

• Molecule 1: GREEN FLUORESCENT PROTEIN



4 Data and refinement statistics

Property	Value	Source
Space group	P 21 21 21	Depositor
Cell constants a, b, c, α , β , γ	51.93Å 62.90Å 69.17Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	24.00 – 2.25 24.31 – 2.00	Depositor EDS
% Data completeness (in resolution range)	96.0 (24.00-2.25) 88.2 (24.31-2.00)	Depositor EDS
R_{merge}	0.06	Depositor
R_{sym}	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ ¹	1.64 (at 1.99Å)	Xtriage
Refinement program	TNT 5F	Depositor
R, R_{free}	0.193 , (Not available) 0.198 , (Not available)	Depositor DCC
R_{free} test set	No test flags present.	wwPDB-VP
Wilson B-factor (Å ²)	33.4	Xtriage
Anisotropy	0.381	Xtriage
Bulk solvent k_{sol} (e/Å ³), B_{sol} (Å ²)	0.28 , 130.0	EDS
L-test for twinning ²	$\langle L \rangle = 0.50$, $\langle L^2 \rangle = 0.33$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o, F_c correlation	0.95	EDS
Total number of atoms	1777	wwPDB-VP
Average B, all atoms (Å ²)	43.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 7.70% of the height of the origin peak. No significant pseudotranslation is detected.*

¹Intensities estimated from amplitudes.

²Theoretical values of $\langle |L| \rangle$, $\langle L^2 \rangle$ for acentric reflections are 0.5, 0.333 respectively for untwinned datasets, and 0.375, 0.2 for perfectly twinned datasets.

5 Model quality

5.1 Standard geometry

Bond lengths and bond angles in the following residue types are not validated in this section: CRO

The Z score for a bond length (or angle) is the number of standard deviations the observed value is removed from the expected value. A bond length (or angle) with $|Z| > 5$ is considered an outlier worth inspection. RMSZ is the root-mean-square of all Z scores of the bond lengths (or angles).

Mol	Chain	Bond lengths		Bond angles	
		RMSZ	$\# Z > 5$	RMSZ	$\# Z > 5$
1	A	0.88	8/1733 (0.5%)	1.49	28/2348 (1.2%)

Chiral center outliers are detected by calculating the chiral volume of a chiral center and verifying if the center is modelled as a planar moiety or with the opposite hand. A planarity outlier is detected by checking planarity of atoms in a peptide group, atoms in a mainchain group or atoms of a sidechain that are expected to be planar.

Mol	Chain	#Chirality outliers	#Planarity outliers
1	A	1	0

All (8) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	A	111	GLU	CD-OE2	6.23	1.32	1.25
1	A	95	GLU	CD-OE2	6.04	1.32	1.25
1	A	115	GLU	CD-OE2	5.65	1.31	1.25
1	A	213	GLU	CD-OE2	5.50	1.31	1.25
1	A	5	GLU	CD-OE2	5.37	1.31	1.25
1	A	222	GLU	CD-OE2	5.30	1.31	1.25
1	A	32	GLU	CD-OE2	5.28	1.31	1.25
1	A	17	GLU	CD-OE2	5.21	1.31	1.25

All (28) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	A	88	MET	C-N-CD	-29.35	56.03	120.60
1	A	89	PRO	N-CA-CB	9.39	114.57	103.30
1	A	173	ASP	CB-CG-OD2	-8.30	110.83	118.30
1	A	82	ASP	CB-CG-OD2	-8.07	111.04	118.30
1	A	197	ASP	CB-CG-OD2	-7.16	111.86	118.30

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Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	A	88	MET	O-C-N	6.74	133.91	121.10
1	A	180	ASP	CB-CG-OD2	-6.71	112.26	118.30
1	A	168	ARG	N-CA-CB	-6.68	98.57	110.60
1	A	21	ASP	CB-CG-OD2	-6.60	112.36	118.30
1	A	216	ASP	CB-CG-OD2	-6.54	112.42	118.30
1	A	173	ASP	CB-CG-OD1	6.41	124.07	118.30
1	A	106	TYR	CB-CG-CD1	6.32	124.79	121.00
1	A	216	ASP	CB-CG-OD1	6.15	123.84	118.30
1	A	109	ARG	NE-CZ-NH1	6.13	123.36	120.30
1	A	106	TYR	CB-CG-CD2	-6.12	117.33	121.00
1	A	5	GLU	N-CA-CB	6.09	121.56	110.60
1	A	210	ASP	CB-CG-OD2	-6.03	112.88	118.30
1	A	102	ASP	CB-CG-OD1	6.02	123.72	118.30
1	A	88	MET	C-N-CA	5.99	147.15	122.00
1	A	76	ASP	CB-CG-OD2	-5.86	113.03	118.30
1	A	102	ASP	CB-CG-OD2	-5.81	113.07	118.30
1	A	88	MET	N-CA-CB	5.76	120.96	110.60
1	A	197	ASP	CB-CG-OD1	5.71	123.44	118.30
1	A	82	ASP	CB-CG-OD1	5.68	123.41	118.30
1	A	21	ASP	CB-CG-OD1	5.62	123.36	118.30
1	A	103	ASP	CB-CG-OD2	-5.30	113.53	118.30
1	A	36	ASP	CB-CG-OD2	-5.27	113.56	118.30
1	A	180	ASP	CB-CG-OD1	5.07	122.86	118.30

All (1) chirality outliers are listed below:

Mol	Chain	Res	Type	Atom
1	A	88	MET	CA

There are no planarity outliers.

5.2 Too-close contacts

In the following table, the Non-H and H(model) columns list the number of non-hydrogen atoms and hydrogen atoms in the chain respectively. The H(added) column lists the number of hydrogen atoms added and optimized by MolProbity. The Clashes column lists the number of clashes within the asymmetric unit, whereas Symm-Clashes lists symmetry related clashes.

Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
1	A	1715	0	1575	69	0
2	A	62	0	0	6	0
All	All	1777	0	1575	69	0

The all-atom clashscore is defined as the number of clashes found per 1000 atoms (including hydrogen atoms). The all-atom clashscore for this structure is 21.

All (69) close contacts within the same asymmetric unit are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:198:ASN:HD22	1:A:198:ASN:N	1.75	0.85
1:A:23:ASN:ND2	1:A:130:PHE:HB2	1.92	0.82
1:A:43:THR:O	1:A:44:LEU:HD23	1.83	0.79
1:A:33:GLY:HA3	1:A:44:LEU:CD2	2.13	0.79
1:A:203:THR:HG23	1:A:224:VAL:HG22	1.64	0.78
1:A:96:ARG:HG3	1:A:183:GLN:HB2	1.64	0.77
1:A:198:ASN:N	1:A:198:ASN:ND2	2.34	0.75
1:A:199:HIS:HB3	1:A:229:ILE:HG13	1.69	0.73
1:A:75:PRO:HA	2:A:396:HOH:O	1.89	0.72
1:A:96:ARG:CG	1:A:183:GLN:HB2	2.20	0.71
1:A:210:ASP:OD1	1:A:211:PRO:HD2	1.91	0.71
1:A:4:GLY:O	1:A:7:LEU:HB2	1.91	0.70
1:A:167:ILE:O	1:A:178:LEU:HD22	1.95	0.67
1:A:198:ASN:O	1:A:229:ILE:HD11	1.95	0.66
1:A:33:GLY:HA3	1:A:44:LEU:HD22	1.77	0.66
1:A:109:ARG:HH11	1:A:109:ARG:HG2	1.61	0.65
1:A:198:ASN:ND2	1:A:198:ASN:H	1.94	0.65
1:A:152:ILE:O	1:A:198:ASN:HA	1.98	0.64
1:A:33:GLY:HA3	1:A:44:LEU:HD23	1.78	0.64
1:A:23:ASN:HD22	1:A:137:LEU:HD11	1.64	0.62
1:A:103:ASP:OD1	1:A:104:GLY:N	2.33	0.60
1:A:103:ASP:OD1	1:A:130:PHE:HA	2.02	0.59
1:A:109:ARG:HH11	1:A:109:ARG:CG	2.16	0.58
1:A:96:ARG:HD2	2:A:308:HOH:O	2.04	0.57
1:A:141:LEU:HD13	1:A:169:HIS:HB3	1.86	0.57
1:A:27:PHE:CD1	1:A:54:PRO:HG2	2.40	0.56
1:A:96:ARG:HG2	1:A:182:TYR:O	2.05	0.56
1:A:56:PRO:HD2	1:A:136:ILE:HG23	1.87	0.55
1:A:46:PHE:O	1:A:217:HIS:HB2	2.07	0.54
1:A:75:PRO:HB2	1:A:77:HIS:CE1	2.43	0.54
1:A:66:CRO:CE1	1:A:203:THR:HG21	2.37	0.54
1:A:93:VAL:O	1:A:185:ASN:HA	2.08	0.53
1:A:154:ALA:HB2	1:A:196:PRO:HD2	1.89	0.53
1:A:100:PHE:HB2	1:A:104:GLY:O	2.10	0.52
1:A:40:GLY:O	1:A:223:PHE:HA	2.09	0.52
1:A:44:LEU:HD12	1:A:46:PHE:CZ	2.45	0.52
1:A:45:LYS:HE2	1:A:47:ILE:HD11	1.91	0.51

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:135:ASN:HA	1:A:140:LYS:CG	2.42	0.49
1:A:22:VAL:O	1:A:25:HIS:HB2	2.14	0.48
1:A:157:GLN:C	1:A:159:ASN:H	2.17	0.48
1:A:164:ASN:O	1:A:165:PHE:HB3	2.13	0.48
1:A:103:ASP:CG	1:A:104:GLY:H	2.18	0.47
1:A:23:ASN:HD22	1:A:130:PHE:HB2	1.75	0.47
1:A:166:LYS:CD	1:A:178:LEU:HD11	2.44	0.47
1:A:199:HIS:CB	1:A:229:ILE:HG13	2.41	0.47
1:A:23:ASN:HD21	1:A:130:PHE:HB2	1.78	0.47
1:A:135:ASN:N	2:A:307:HOH:O	2.35	0.46
1:A:198:ASN:HB3	2:A:366:HOH:O	2.15	0.46
1:A:131:LYS:O	1:A:137:LEU:HB2	2.16	0.46
1:A:125:LEU:HG	1:A:126:LYS:N	2.30	0.45
1:A:27:PHE:CD1	1:A:54:PRO:CG	2.99	0.45
1:A:81:HIS:CE1	1:A:197:ASP:H	2.35	0.45
1:A:213:GLU:OE2	1:A:215:ARG:NE	2.47	0.45
1:A:195:LEU:HD22	1:A:195:LEU:HA	1.72	0.45
1:A:210:ASP:HA	1:A:211:PRO:HD3	1.73	0.44
1:A:78:MET:SD	1:A:229:ILE:HB	2.57	0.44
1:A:135:ASN:HB2	2:A:307:HOH:O	2.17	0.43
1:A:98:ILE:HD13	1:A:181:HIS:NE2	2.32	0.43
1:A:153:MET:HG2	1:A:153:MET:O	2.16	0.43
1:A:109:ARG:CG	1:A:109:ARG:NH1	2.80	0.43
1:A:29:VAL:HA	1:A:47:ILE:O	2.19	0.43
1:A:137:LEU:HA	1:A:137:LEU:HD23	1.77	0.43
1:A:7:LEU:HD21	1:A:89:PRO:HG3	2.00	0.42
1:A:66:CRO:N2	1:A:66:CRO:OG1	2.40	0.42
1:A:208:SER:HB3	2:A:371:HOH:O	2.18	0.42
1:A:44:LEU:HD12	1:A:46:PHE:HZ	1.84	0.40
1:A:158:LYS:O	1:A:159:ASN:C	2.60	0.40
1:A:47:ILE:HG22	1:A:48:CYS:N	2.36	0.40
1:A:133:ASP:O	1:A:140:LYS:NZ	2.54	0.40

There are no symmetry-related clashes.

5.3 Torsion angles ⓘ

5.3.1 Protein backbone ⓘ

In the following table, the Percentiles column shows the percent Ramachandran outliers of the chain as a percentile score with respect to all X-ray entries followed by that with respect to entries

of similar resolution.

The Analysed column shows the number of residues for which the backbone conformation was analysed, and the total number of residues.

Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percentiles
1	A	220/236 (93%)	203 (92%)	14 (6%)	3 (1%)	12 8

All (3) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	89	PRO
1	A	158	LYS
1	A	157	GLN

5.3.2 Protein sidechains ⓘ

In the following table, the Percentiles column shows the percent sidechain outliers of the chain as a percentile score with respect to all X-ray entries followed by that with respect to entries of similar resolution.

The Analysed column shows the number of residues for which the sidechain conformation was analysed, and the total number of residues.

Mol	Chain	Analysed	Rotameric	Outliers	Percentiles
1	A	165/206 (80%)	143 (87%)	22 (13%)	4 2

All (22) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	5	GLU
1	A	7	LEU
1	A	9	THR
1	A	15	LEU
1	A	19	ASP
1	A	30	SER
1	A	80	ARG
1	A	83	PHE
1	A	86	SER
1	A	109	ARG
1	A	125	LEU
1	A	153	MET
1	A	168	ARG
1	A	176	VAL

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Mol	Chain	Res	Type
1	A	178	LEU
1	A	180	ASP
1	A	194	LEU
1	A	195	LEU
1	A	198	ASN
1	A	203	THR
1	A	225	THR
1	A	229	ILE

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (3) such sidechains are listed below:

Mol	Chain	Res	Type
1	A	23	ASN
1	A	170	ASN
1	A	198	ASN

5.3.3 RNA ⓘ

There are no RNA molecules in this entry.

5.4 Non-standard residues in protein, DNA, RNA chains ⓘ

1 non-standard protein/DNA/RNA residue is modelled in this entry.

In the following table, the Counts columns list the number of bonds (or angles) for which Mogul statistics could be retrieved, the number of bonds (or angles) that are observed in the model and the number of bonds (or angles) that are defined in the Chemical Component Dictionary. The Link column lists molecule types, if any, to which the group is linked. The Z score for a bond length (or angle) is the number of standard deviations the observed value is removed from the expected value. A bond length (or angle) with $|Z| > 2$ is considered an outlier worth inspection. RMSZ is the root-mean-square of all Z scores of the bond lengths (or angles).

Mol	Type	Chain	Res	Link	Bond lengths			Bond angles		
					Counts	RMSZ	$\# Z > 2$	Counts	RMSZ	$\# Z > 2$
1	CRO	A	66	1	23,23,24	2.49	9 (39%)	27,32,34	1.82	8 (29%)

In the following table, the Chirals column lists the number of chiral outliers, the number of chiral centers analysed, the number of these observed in the model and the number defined in the Chemical Component Dictionary. Similar counts are reported in the Torsion and Rings columns. '-' means no outliers of that kind were identified.

Mol	Type	Chain	Res	Link	Chirals	Torsions	Rings
1	CRO	A	66	1	-	0/12/31/32	0/2/2/2

All (9) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	A	66	CRO	OH-CZ	-4.34	1.26	1.37
1	A	66	CRO	CG2-CB2	-2.55	1.41	1.46
1	A	66	CRO	CA3-N3	-2.51	1.42	1.47
1	A	66	CRO	C1-N3	2.04	1.40	1.37
1	A	66	CRO	CD1-CG2	2.95	1.45	1.39
1	A	66	CRO	CD2-CG2	2.96	1.45	1.39
1	A	66	CRO	CE2-CZ	3.93	1.46	1.38
1	A	66	CRO	CE1-CZ	5.42	1.49	1.38
1	A	66	CRO	CB2-CA2	5.91	1.40	1.35

All (8) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	A	66	CRO	CA1-C1-N3	-3.68	120.33	124.75
1	A	66	CRO	CE2-CZ-CE1	-2.93	114.61	119.75
1	A	66	CRO	CA3-N3-C1	-2.23	124.59	127.20
1	A	66	CRO	CA3-N3-C2	2.20	128.89	123.88
1	A	66	CRO	CG2-CB2-CA2	2.33	132.76	130.10
1	A	66	CRO	CD2-CE2-CZ	2.45	122.61	119.88
1	A	66	CRO	CA2-C2-N3	3.41	104.77	103.30
1	A	66	CRO	N3-C1-N2	4.07	114.27	111.45

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

1 monomer is involved in 2 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
1	A	66	CRO	2	0

5.5 Carbohydrates ⓘ

There are no carbohydrates in this entry.

5.6 Ligand geometry

There are no ligands in this entry.

5.7 Other polymers

There are no such residues in this entry.

5.8 Polymer linkage issues

There are no chain breaks in this entry.

6 Fit of model and data [i](#)

6.1 Protein, DNA and RNA chains [i](#)

In the following table, the column labelled ‘#RSRZ> 2’ contains the number (and percentage) of RSRZ outliers, followed by percent RSRZ outliers for the chain as percentile scores relative to all X-ray entries and entries of similar resolution. The OWAB column contains the minimum, median, 95th percentile and maximum values of the occupancy-weighted average B-factor per residue. The column labelled ‘Q< 0.9’ lists the number of (and percentage) of residues with an average occupancy less than 0.9.

Mol	Chain	Analysed	<RSRZ>	#RSRZ>2	OWAB(Å ²)	Q<0.9
1	A	224/236 (94%)	0.47	21 (9%) 8 9	26, 41, 63, 81	5 (2%)

All (21) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	211	PRO	5.3
1	A	229	ILE	3.3
1	A	60	LEU	3.1
1	A	64	PHE	3.1
1	A	61	VAL	3.1
1	A	84	PHE	3.0
1	A	51	GLY	2.8
1	A	11	VAL	2.7
1	A	69	GLN	2.7
1	A	153	MET	2.7
1	A	70	CYS	2.6
1	A	160	GLY	2.6
1	A	136	ILE	2.5
1	A	68	VAL	2.5
1	A	191	GLY	2.5
1	A	130	PHE	2.3
1	A	217	HIS	2.3
1	A	133	ASP	2.2
1	A	63	THR	2.2
1	A	22	VAL	2.1
1	A	189	GLY	2.1

6.2 Non-standard residues in protein, DNA, RNA chains [i](#)

In the following table, the Atoms column lists the number of modelled atoms in the group and the number defined in the chemical component dictionary. The B-factors column lists the minimum,

median, 95th percentile and maximum values of B factors of atoms in the group. The column labelled 'Q< 0.9' lists the number of atoms with occupancy less than 0.9.

Mol	Type	Chain	Res	Atoms	RSCC	RSR	B-factors(Å ²)	Q<0.9
1	CRO	A	66	22/23	0.90	0.26	20,31,41,48	0

6.3 Carbohydrates [i](#)

There are no carbohydrates in this entry.

6.4 Ligands [i](#)

There are no ligands in this entry.

6.5 Other polymers [i](#)

There are no such residues in this entry.